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Studies the residual effect of six herbicides applied to the minimum tillage non-puddled transplanted winter and summer rice in Bangladesh

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Abstract:

The poisonous effect of herbicide residues on the succeeding crops is one of the principal concerns against the safe use of herbicides for controlling weeds. Acute labor crisis for crop production pushes farmers to adopt minimum tillage non-puddled (MTNP) rice cultivation in Bangladesh. This on-farm research on plant bioassay was conducted at the Mymensingh region of Bangladesh during October–December in 2016 and 2017 year. Here, we studied any residual effect of six herbicides *viz.*, glyphosate, pendimethalin, ethoxysulfuron-ethyl, isoproturon, fenoxaprop-p-ethyl, and carfentrazone-ethyl + isoproturon on the succeeding mustard in a winter rice-summer rice-mustard rotation. These herbicides were applied in 10 different combinations for controlling weeds of MTNP winter and summer rice for two years. Immediately after the harvest of summer rice, the indicator crop mustard was planted on the same plots of the respective treatments of the previous MTNP rice experiment. Data of a two-year experiment revealed that, after 25 days of planting, the plant population, length of seedling root and shoot, and seedling biomass did not vary significantly across the treatments. Moreover, leaf chlorophyll content in all the treatments was statistically identical. Furthermore, an excellent growth mustard plant without any sign of phytotoxicity was observed in all treatments. Hence, it could be concluded that herbicides used for controlling weeds in MTNP rice did not retain any residues in soil enough to hamper the growth and development of succeeding mustard in winter rice-summer rice-mustard rotation Bangladesh.

Keywords: bioassay; herbicide residue; toxicity; chlorophyll; phytotoxicity

Introduction:

In Bangladesh, rice (*Oryza sativa* L.) is conventionally cultivated by transplanting seedlings into puddled soil, typically for ease of crop establishment and weed control [1]. But rice can be grown by transplanting them into minimum tillage non-puddled (MTNP) soils without any yield penalty but with additional benefits of saving land preparation costs, fuel energy requirement, labor, and irrigation water [2, 3]. However, severe weed infestation has been argued against the widespread adoption of MTNP rice cultivation [4, 5]. As a result, farmers are advised to do hand weeding up to six times throughout the rice-growing season to maintain weeds below the economic threshold level in this practice [6]. Due to the agricultural laborers' crisis and high wage rate, herbicides are being quickly embraced in countries with a manpower shortage for weeding [7, 8]. Recent advancements in broad-spectrum herbicides may offer an opportunity to manage weeds more successfully in non-puddled rice transplanting systems [9].

Unfortunately, the repeated use of these chemicals may lead to persists residues of herbicides in the soil [10]. Wyk and Reinhardt [11] discovered an excessive quantity of imazethapyr residue harmed corn planted after soybean. If herbicide residues remain in the soil, they may decrease the performance of subsequent crops [12]. Sulfonylurea herbicide residues even at low concentrations in soil may damage rotating crops [13, 14]. However, farmers often apply herbicides without understanding or evaluating the herbicide's residual impact on following crops.

Furthermore, minimal study on the residual effects of herbicides on the following crops has been conducted in Bangladesh. In general, a soil chemical test or bioassay may be performed to assess the residual herbicide content in the soil [15]. However, chemical analysis is prohibitively expensive, and therefore a plant bioassay in the field may be used to predict the presence of herbicides in soil. While a bioassay does not quantify the herbicide residue in the soil but shows whether there is enough residue in the soil to harm a succeeding crop practically. The bioassay in a similar field of previous herbicide-treated crops is more convenient and indicates the residual impact of herbicides in the field scenario. With this point of view, bioassay research was performed on-farm at the farmers' field immediately after harvest of MTNP summer rice to determine the residual impact of rice herbicides on subsequent mustard under winter rice- summer rice-mustard cropping system in Bangladesh.

Materials and Methods:
Location and tenure:

This on-farm experiment was conducted at the farmers' field located at the Mymensingh region of Bangladesh (N: 24°75', E: 90°50') from October–March in 2016 and 2017 years under the mustard-winter rice-summer rice cropping pattern.

Soil condition:

The field was a well-drained medium medium-high land with sandy clay loam soil with sand, silt, and Clay @ 50, 23, and 27%, respectively, and soil pH of 7.2.

Climatic statement:

The region gets an average annual rainfall of 178 millimeters, with about 93% of it falling between May and September (Figure 1). Total rain was greatest during the summer rice season and lowest during the winter rice season in both years. Occasionally, the highest average temperature was about 29°C in April–May, while the lowest temperature was approximately 13°C in January. In both years, the months of October–November, and March had the most sunshine hours.

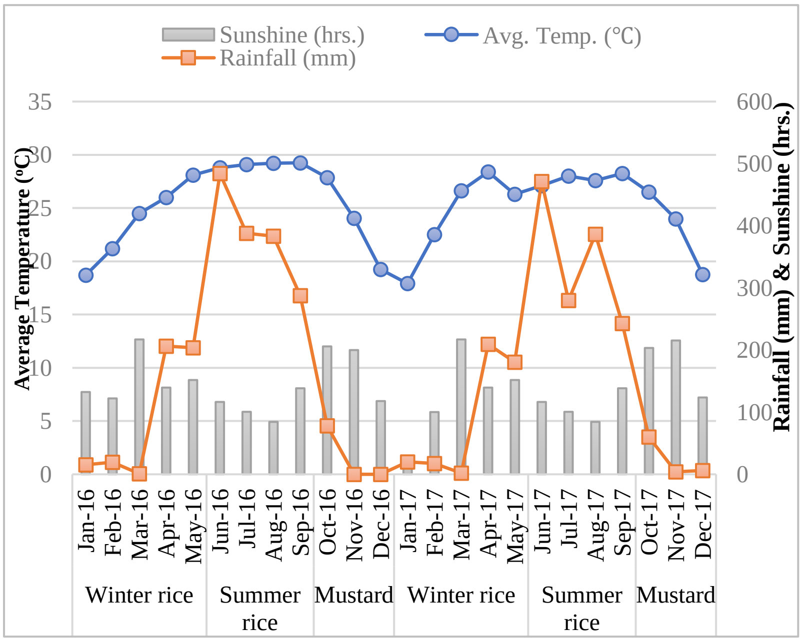

Figure 1: Climatic condition of the on-farm experimental site at the Mymensingh region of Bangladesh during 2016 and 2017.

MTNP rice experiment:

Winter rice (*Oryza sativa* L.) during January–May and summer rice during June–September was grown under MTNP system continuously two years during 2016 and 2017. The MTNP rice experiment used a four-replicated randomized complete block design. The unit plots were 9 m × 5 m in size. The MTNP land was prepared in a single pass operation, using the Versatile Multi-crop Planter (VMP) machine. Six rows each of 6 cm broad and 5 cm deep was made at a time. Total ten combinations of six herbicides (Table 1) were applied in winter and summer rice per the recommended rates and time (Table 2). Glyphosate and pendimethalin were applied 3 days before and after planting, respectively. Rest of all other were applied at 25 days after planting. Among them only ethoxysulfuron-ethyl was applied in standing water and rest of all were applied in field capacity condition.

Table 1: Treatments using different herbicides in MTNP rice experiments for
weed control

Legends	**Treatments**
T_0	Control
T_1	Glyphosate
T_2	Glyphosate followed by (*fb)* Pendimethalin
T_3	Glyphosate *fb* Ethoxysulfuron-ethyl
T_4	Glyphosate *fb* Carfentrazone-ethyl+Isoproturon
T_5	Glyphosate *fb* Isoproturon
T_6	Glyphosate *fb* Fenoxaprop-p-ethyl
T_7	Glyphosate *fb* Pendimethalin *fb* Ethoxysulfuron-ethyl
T_8	Glyphosate fb Pendimethalin fb Carfentrazone-ethyl+Isoproturon
T_9	Glyphosate *fb* Pendimethalin *fb* Isoproturon
T_{10}	Glyphosate *fb* Pendimethalin *fb* Fenoxaprop-p-ethyl

Table 2: Statements of herbicides used in MTNP rice

Name of herbicides	**Chemical group**	**Rate (a.i. ha^{-1})**
Glyphosate	Phosphonic acid	9 L
Pendimethalin	Dinitroaniline	11 L
Ethoxysulfuron-ethyl	Sulfonylurea	667 g
Carfentrazone-ethyl	Triazolinone	2.5 kg
Isoproturon	Phenylurea	3.4 L
Fenoxaprop-p-ethyl	Aryloxy-phenoxy-propionate	7.2 L

*a.i. = active ingredient

Bioassay experiment:

The research used the bioassay technique to determine the residual impact of herbicides applied to winter and summer rice on subsequent mustard (*Brassica napus* L.). Mustard was grown from October–December. On the relevant plots, planting of 7 kg, ha^{-1} seeds were done using VMP on October 05 in 2016 and 2017, immediately after the harvest of summer rice. Light watering was done after planting for optimal germination. During the residual impact research period, no fertilizer was applied in the field. Before planting in experimental plots, sample seed germination was examined in the laboratory, and >80% germination was reported. Weeds were maintained below the economic threshold level by manual weeding throughout the growing season [16].

Measurements:

A 1.0 m × 1.0 m quadrat was used to record the plant population m^{-2}. The quadrat was randomly put in three locations within each plot. Plants were counted inside the quadrat, and an average of three quadrates was reported. The length of root and shoot and the biomass of 25 days aged seedlings was determined by examining the biomass after 72 hours of drying at 70°C of randomly selected ten plants. The chlorophyll content of the leaves using *SPAD 502 Plus Chlorophyll Meter* from the young, tender leaf of these plants.
Phyto-toxicity of herbicide on mustard and crop vigor of mustard was assessed visually four times at 15 days of interval up to 60 DAS following the toxicity grading of IRRI [17] (Table 3) and crop vigor scale [18] as of; 1: Poor, 2: Fair, 3: Good and 4: Excellent.

Table 3: Phyto-toxicity scoring

Toxicity	Rating
Normal growth: non-toxic	1
Slightly toxic: Injury/discoloration recoverable	2
Moderately toxic: Some stunting/discoloration recoverable	3
Severely toxic: Stand loss irrecoverable	4
Toxic (Plant kill): Total damage	5

Data analysis:

The data were analyzed using the statistical software STAR following analysis of variance, and treatment means were separated using the Duncans' Multiple Range Test at a significance level of 5%.

Results:

Effect on plant population:

The plant population of mustard m^{-2} areas at 25 DAS did not vary significantly (p>5%) at both 2016 and 2017 years by the residues of six MTNP rice herbicides (Figure 2). We recorded >80 plants m^{-2} across all the treatments in both years. Data implies that the previously applied herbicides did persist in the soil enough to hamper the germination capacity of mustard.

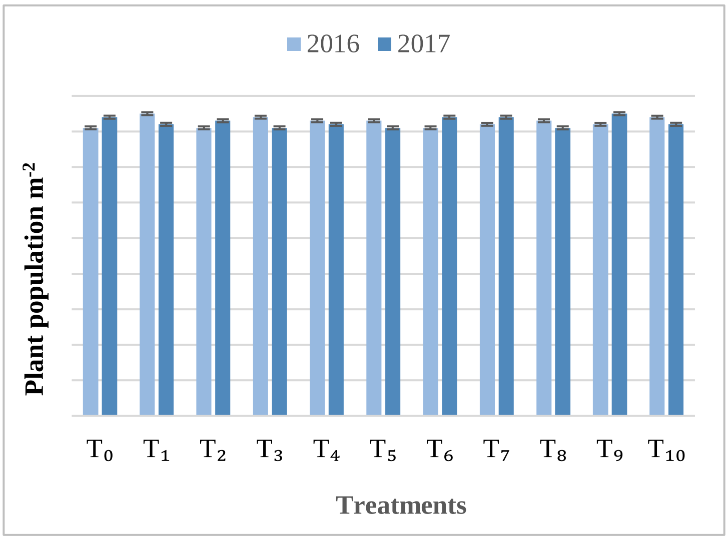

Figure 2: Residual effect of herbicides on the plant population of mustard at p > 5% level. T_0 = Control, T_1 = Glyphosate, T_2 = Glyphosate *followed by* (*fb*) Pendimethalin, T_3 = Glyphosate *fb* Ethoxysulfuron-ethyl, T_4 = Glyphosate *fb* Carfentrazone-ethyl+Isoproturon, T_5 = Glyphosate *fb* Isoproturon, T_6 = Glyphosate *fb* Fenoxaprop-p-ethyl, T_7 = Glyphosate *fb* Pendimethalin *fb* Ethoxysulfuron-ethyl, T_8 = Glyphosate *fb* Pendimethalin *fb* Carfentrazone-ethyl+Isoproturon, T_9 = Glyphosate *fb* Pendimethalin *fb* Isoproturon, T_{10} = Glyphosate *fb* Pendimethalin *fb* Fenoxaprop-p-ethyl

Effect on the length of root and shoot at 25 DAS:

Data presented in Table 4 indicated a statistically non-significant (p > 5%) effect of previously used herbicides on the root and shoot lengths of mustard at 25 DAS in both 2016 and 2017 year. None of the other treatments relative to Control did inhibit the root and shoot growth. Results revealed no residual effect of MTNP rice herbicides on the root and shoot development of succeeding mustard.

Table 4: Residual effect of herbicides on the root and shoot length of mustard

Treatments	Root length (cm)		Shoot length (cm)	
	2016	**2017**	**2016**	**2017**
T_0	6.41	5.26	16.82	18.5
T_1	6.45	5.32	22.01	22.5
T_2	7.39	5.68	22.72	20.6
T_3	7.42	5.58	21.30	24.0
T_4	6.62	6.06	21.22	20.5
T_5	7.42	5.91	20.55	22.5
T_6	6.39	5.88	22.71	23.5
T_7	6.45	6.32	22.23	25.0
T_8	7.43	5.93	18.61	21.0
T_9	6.56	5.97	20.27	23.3
T_{10}	7.47	5.87	20.93	20.5
STDV	0.09	0.30	2.06	1.83
CV	1.25	5.27	10.32	8.36
SE	0.03	0.09	2.98	2.41

STDV = Standard Deviation, CV = Co-efficient of variance, SE = Standard error of mean difference
T_0 = Control, T_1 = Glyphosate, T_2 = Glyphosate *followed by* (*fb*) Pendimethalin, T_3 = Glyphosate *fb* Ethoxysulfuron-ethyl, T_4 = Glyphosate *fb* Carfentrazone-ethyl+Isoproturon, T_5 = Glyphosate *fb* Isoproturon, T_6 = Glyphosate *fb* Fenoxaprop-p-

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Effect on the leaf chlorophyll content at 25 DAS:

The chlorophyll contents of mustard leaves based on the SPAD meter reading reported a non-significant ($p > 5\%$) variation by the carryover effect of rice herbicides (Figure 3). Data reveal that six herbicides in 10 combinations used in earlier MTNP winter and summer rice to control weeds does not persist in the soil to hamper the leaf chlorophyll contents of succeeding mustard.

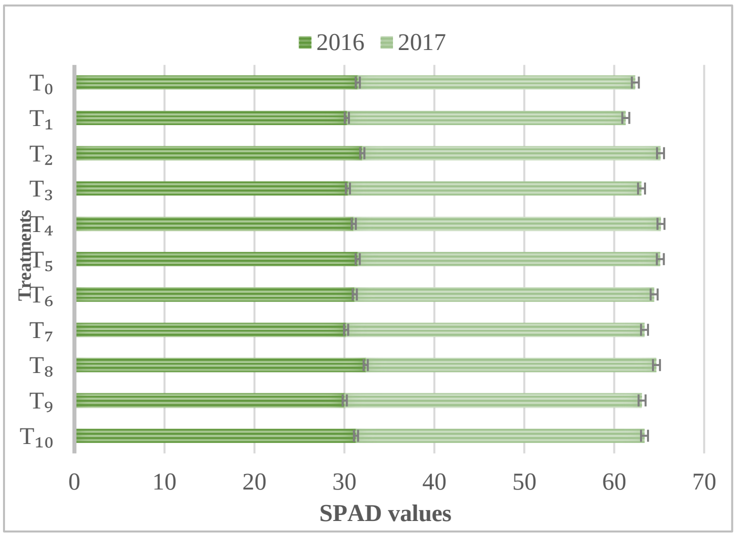

Figure 3: Residual effect of herbicides on the chlorophyll content of mustard leaves at $p > 5\%$ level. T_0 = Control, T_1 = Glyphosate, T_2 = Glyphosate *followed by* (*fb*) Pendimethalin, T_3 = Glyphosate *fb* Ethoxysulfuron-ethyl, T_4 = Glyphosate *fb* Carfentrazone-ethyl+Isoproturon, T_5 = Glyphosate *fb* Isoproturon, T_6 = Glyphosate *fb* Fenoxaprop-p-ethyl, T_7 = Glyphosate *fb* Pendimethalin *fb* Ethoxysulfuron-ethyl, T_8 = Glyphosate *fb* Pendimethalin *fb* Carfentrazone-ethyl+Isoproturon, T_9 = Glyphosate *fb* Pendimethalin *fb* Isoproturon, T_{10} = Glyphosate *fb* Pendimethalin *fb* Fenoxaprop-p-ethyl

Effect on seedling biomass of mustard at 25 DAS:

Statically similar ($p > 5\%$) biomass of the 25 days aged seedlings of mustard at all the treatments relative to Control was recorded in both years in this study (Table 5).

Table 5: Residual effect of herbicides on seedling biomass of mustard at 25 DAS

Treatments	**Plant biomass (g 10 plant^{-1})**	
	2016	**2017**
T_0	1.40	1.31
T_1	1.47	1.32
T_2	1.46	1.29
T_3	1.43	1.36
T_4	1.41	1.42
T_5	1.46	1.40
T_6	1.47	1.39
T_7	1.46	1.43
T_8	1.51	1.47
T_9	1.53	1.49
T_{10}	1.42	1.41
STDV	0.12	0.11
CV	8.24	7.34
SE	2.27	2.11

STDV = Standard Deviation, CV = Co-efficient of variance, SE = Standard error of mean difference

T_0 = Control, T_1 = Glyphosate, T_2 = Glyphosate *fb* Pendimethalin, T_3 = Glyphosate *fb* Ethoxysulfuron-ethyl, T_4 = Glyphosate *fb* Carfentrazone-ethyl+Isoproturon, T_5 = Glyphosate *fb* Isoproturon, T_6 = Glyphosate *fb* Fenoxaprop-p-ethyl, T_7 = Glyphosate *fb* Pendimethalin *fb* Ethoxysulfuron-ethyl, T_8 = Glyphosate *fb* Pendimethalin *fb* Carfentrazone-ethyl+Isoproturon, T_9 = Glyphosate *fb* Pendimethalin *fb* Isoproturon, T_{10} = Glyphosate *fb* Pendimethalin *fb* Fenoxaprop-p-ethyl

Visual scoring of phytotoxicity and crop vigor:

The visual observation scoring of toxicity symptoms on the morphology scored "1" and crop vigor scored "4" (data not shown), indicating an excellent crop growth without any poisonous symptoms relative to Control treatment. Such results suggest no carryover effect of previously used six MTNP rice herbicides on the succeeding mustard.

Discussion:

The current two-year on-farm research investigated any potential persistence impact of six rice herbicides (glyphosate, pendimethalin, ethoxysulfuron-ethyl, carfentrazone-ethyl + isoproturon, isoproturon, and fenoxaprop-p-ethyl) on the indicator crop plant mustard. The results demonstrated applied rice herbicides in 10 different combinations had no harmful effect on subsequent mustard plant population, length of shoot and root, leaf chlorophyll content and seedling biomass. Moreover, no toxic symptoms were observed visually on healthy plant growth across all the treatments. The finding of prior research agrees with our results showing that herbicides used in the preceding wheat crop did not affect maize germination [19, 20]. Additionally, they noticed no apparent phototoxicity on mustard by the residues of imazethapyr + pendimethalin applied to black gram. Another research has shown that herbicides applied to onions [21] and peanuts [22] did not substantially impact the germination of subsequent sorghum and wheat, and gram. Khokhar and Charak [23] also found that herbicides sprayed to wheat had no discernible impact on the germination of the subsequent maize, green gram, and cucumber. The explanation for this may be linked to the degradation of all herbicides in soil [24] which is related to the half-life of the herbicides examined. For example, half-life (days) of glyphosate: 30–32 days [25], pendimethalin: 25–35 days [26], ethoxysulfuron: 60 days [27], carfentrazone-ethyl: 3.8–5.8 hours only [28], isoproturon: 24 days [29] and fenoxaprop-p-ethyl: 1.45–2.30 days [30]. On the other hand, mustard required about 90 days to harvest in our prior study. Thus, as Parthipan et al. [31] and Yazdanpak et al. [32] indicated, there was little chance of these herbicides persisting in the soil until the next crop growing season. The unaffected germination rate might have influenced the to obtain a similar plant population of succeeding mustard across all treatments in this study.

At 25 DAS, the current research discovered no significant impact on the seedlings' shoot and root length and dry matter production. This finding supports Taslima et al. [33]. They disclosed no adverse effects of the residues of eight herbicides (pendimethalin, pretilachlor, triasulfuron, ethoxysulfuron, pyrazosulfuron-ethyl, carfentrazone-ethyl, 2,4-D amine, and carfentrazone-ethyl + isoproturon) on the biomass of succeeding mungbean, sunflower, and jute. The research findings of Yadav and Bhullar [34] also discovered that herbicides applied to soybean had no impact on the dry matter buildup of succeeding wheat, barley, spinach, pea, raya, canola, and sugarbeet due to thoroughly degradation of prior applies herbicides. Further research by Sangeetha et al. [35], Bahrampor and Ziveh [36], and Yadav et al. [37] confirmed no significant residual toxicity in shoot length was seen in the following soybean and wheat treated with herbicides in the prior crop. Similarly, Rathod et al. [21] found that onion herbicide residue had no detrimental effect on the dry matter accumulation of the following sorghum.

Herbicide applied to the MTNP rice did not affect the chlorophyll content of the indicator crop mustard leaves in this research. The excellent plant growth resulted in increased leaf area facilitated to have a higher efficiency of light, water, and nutrients use [38], resulting in increased plant biomass both in the herbicidal and Control treatment in this study. Prior studies assert that herbicide residue had no detrimental impact on phenotypic and genotypic development resulting from the following crops' regular leaf chlorophyll content [31, 35]. Taslima et al. [33] also found an unaffected chlorophyll content in leaves of succeeding sunflower, mungbean, and jute when eight different herbicides were applied to prior wheat.

Any non-toxic effect of herbicide residue on the length of root, leaf chlorophyll content, and plant dry matter might have influenced the non-persistent herbicides in soil. Applied herbicides in may be broken down by the cultural activities of various crops, such as flooding for irrigation and microbial degradation, are the primary mechanisms by which herbicides are dissipated from the soil [39, 40]. Thus, one might argue that many herbicides used for weed management are safe in terms of residual toxicity in soil [41, 42]. The explanation for this may be because the herbicides used have entirely degraded in the soil or that their presence is at a measurable level that does not negatively impact the growth of subsequent crops. Previous investigations concluded that residues of the majority of herbicides remained below the detectable level in the soil after 30–120 days of treatment [26, 43]. Hence, the above-discussed reasons clarify those six herbicides in ten combinations tested in MTNP rice pose no detrimental residual impact on the growth and development of subsequent mustard in Bangladesh.

Conclusion:

The results indicated that ten combinations of six herbicides: glyphosate, pendimethalin, ethoxysulfuron-ethyl, isoproturon, fenoxaprop-p-ethyl, and carfentrazone-ethyl + isoproturon applied to MTNP winter and summer rice had no toxic effect on the plant population, seedling growth in terms of root and shoot length, and biomass and leaf chlorophyll content of succeeding mustard with excellent growth without any phytotoxic symptoms. Thus, the study concluded that herbicides used in preceding MTMP rice are safer for the next seasons' crop cultivation in rotation.

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